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Kidney fatty acid-binding protein: identification as α_{2U}-globulin

Hideka Kimura*+, Shoji Odani*, Jun-ichi Suzuki, Masaai Arakawa+ and Teruo Ono*

*Department of Biochemistry, +Department of Medicine (II), Niigata University School of Medicine, Niigata 951, and Department of Biochemistry, Yamagata University School of Medicine, Yamagata 990-23, Japan

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A fatty acid-binding protein was purified from male rat kidney by the method of Fujii et al. [(1987) Arch. Biochem. Biophys. 254, 552–558]. Based on the close similarity of amino acid composition and the identity of the amino acid sequence in the amino-terminal region, the fatty acid-binding protein was identified as α_{2U}-globulin, which is the major male-specific protein in rat urine. The amino-terminal residue of the isolated kidney protein corresponds to residue 10 of α_{2U}-globulin, suggesting that the protein has been proteolytically processed in the kidney. This fatty acid-binding protein could not be immunologically detected in female rat kidney.

Fatty acid-binding protein; Globulin, α_{2U}-; Male-specific protein; (Rat, Kidney)

1. INTRODUCTION

Fatty acid-binding proteins constitute a family of abundant cytosolic proteins of low molecular masses and are capable of binding fatty acids and other organic anions [1]. They are thought to be responsible for intracellular transport, sequestration and metabolism of long-chain fatty acids [2]. Indeed, these proteins are specifically distributed in such tissues as liver, intestine and heart that are actively involved in fatty acid metabolism [3]. FABPs from these three tissues have been extensively characterized [4–8]. The structural data accumulated have shown that FABPs are structurally related to one another and together with cytosolic proteins such as cellular retinoid-binding proteins and peripheral nerve myelin P2 protein form a protein superfamily [5–8].

Recently, a FABP has been isolated from male rat kidney [9,10]. This protein, termed 'kidney FABP', has a molecular mass of 15.5 kDa, binds an equimolar amount of fatty acids and is distinct from the tissue-specific FABPs so far reported in terms of molecular size and amino acid composition. Moreover, the expression of this protein has been shown to alter in response to drug-induced and genetic hypertension [10–12], suggesting that it plays an important role in renal fatty acid metabolism under pathological conditions as well as normal conditions. However, nothing is as yet known concerning its structure.

We report here that kidney FABP purified from male rat kidney is identical with α_{2U}-globulin, which is the major male-specific protein in adult rat urine.

Correspondence address: H. Kimura, Department of Biochemistry, Niigata University School of Medicine, 1 Asahimachi Dori, Niigata 951, Japan

Abbreviations: FABP, fatty acid-binding protein; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid

2. MATERIALS AND METHODS

2.1. *Materials*

[1-^{14}C]Palmitate (59 mCi/mmol) was purchased from New England Nuclear, Sephadex G-75 from Pharmacia Biotechnology, DE-52 ion-exchange resin from Whatman, and acetonitrile and TFA from Wako Pure Chemical. All other chemicals used were of analytical grade. Polyclonal rabbit anti-kidney FABP antibodies [9] were kindly supplied by Drs S. Fujii and H. Kawaguchi of Hokkaido University.

2.2. *Purification of kidney FABP*

Kidney FABP was purified from kidneys of male Wistar rats

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as described by Fujii et al. [9]. Briefly, the kidney cytosolic fraction was subjected to Sephadex G-75 gel filtration, followed by DE-52 ion-exchange chromatography. The protein thus purified was further applied to a Toyo Soda ODS (octadecylsilica) 120T column (0.45 × 25 cm) pre-equilibrated with 1% acetonitrile in 0.1% TFA. The FABP was then eluted with a linear acetonitrile gradient (1–60%) in 0.1% TFA for 45 min at a flow rate of 1 ml/min. This chromatography was necessary to remove minor contaminants.

2.3. *Amino acid analysis and sequencing*

Protein samples were hydrolyzed in vacuo with 5.7 N HCl at 110°C for 21 h, and the hydrolysates were analyzed with a Hitachi 835 amino acid analyzer. Methionine and cystine were determined after performic acid oxidation [13]. The determination of tryptophan was conducted after hydrolysis with 5.7 N HCl containing 4% thioglycolic acid [14]. The amino-terminal sequence of the FABP was determined by automated Edman degradation in an Applied Biosystem 470A gas-phase sequencer equipped with a 120A phenylthiohydantoin analyzer.

2.4. *Fatty acid-binding assay*

[1-^{14}C]Palmitic acid-binding activity of kidney FABP was measured by the method of Glatz et al. [15]; 1 nmol of protein was incubated with 4 nmol of radioactive palmitic acid.

2.5. *Other method*

Protein concentration was determined by amino acid analysis. Tricine SDS-PAGE was performed as described [16]; using separating gels containing 16.5% acrylamide. Protein bands were stained with Coomassie brilliant blue. Double-immunodiffusion tests were performed by the method of Ouchterlony [17].

3. RESULTS AND DISCUSSION

According to the method of Fujii et al. [9], the cytosolic fraction of male rat kidney was subjected to Sephadex G-75 gel filtration, and the fraction containing proteins of low molecular masses (10–20 kDa) were pooled. The pooled fractions were applied to a DE-52 column and elution was conducted with a linear NaCl gradient (0–0.3 M). As shown in fig.1, three protein peaks, termed P1, P2 and P3, were thereby eluted. Of these peaks, P1 and P3 showed significant [^{14}C]palmitate-binding activities. From its electrophoretic mobility (fig.2) and amino acid composition (not shown), the P1 protein was thought to be similar to heart type FABP [7,8]. On the other hand, the P3 protein exhibited a lower palmitate-binding activity than the P1 protein and had a molecular mass of about 15.5 kDa as estimated by SDS-PAGE (fig.2). After further purification by reversed-phase HPLC, this protein formed a single precipitin line with antibodies to kidney FABP in Ouchterlony test (not

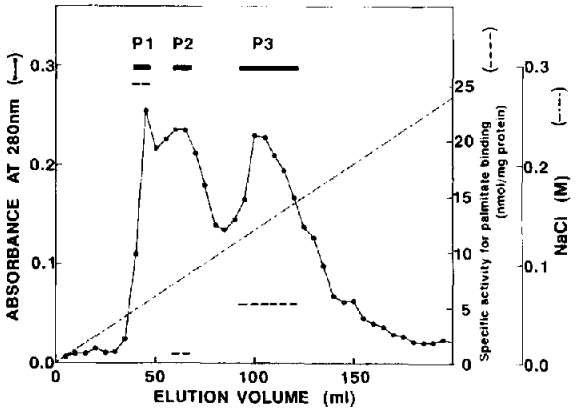

Fig.1. Separation of rat kidney low-molecular-mass protein on DE-52. The fractions containing proteins of low molecular masses (10–20 kDa) of the Sephadex G-75 column were applied to a DE-52 column (1.8 × 10 cm) and eluted with a linear NaCl gradient (0–0.3 M). The horizontal lines marked P1, P2 and P3 denote the fractions which are pooled and studied further. The dashed lines indicate the specific-binding activity for palmitate of the pooled fractions.

shown). Its amino acid composition was very similar to that reported for kidney FABP [10] (table 1). It was, therefore, certain that the P3 protein was kidney FABP. Although the palmitate-binding activity of the P3 protein was lower than that reported for kidney FABP [9], this difference seems to be due to the difference in methodology. While we used Lipidex 5000 for the binding assay [15], the previous workers employed dextran-coated charcoal [9].

To obtain further structural information concerning this kidney FABP, its amino-terminal

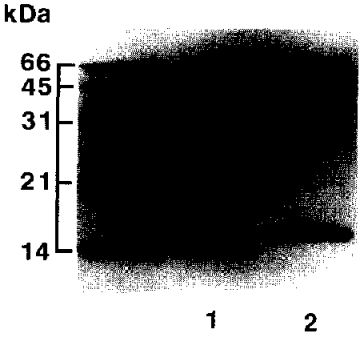

Fig.2. Tricine SDS-PAGE of P1 and P3 proteins of fig.1. Lanes: 1, P1 protein (5 μg); 2, P3 protein (5 μg).

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                          1          10          20
Kidney FABP               L D V A K L N G D W F S I V V A S N K R ·····

                  1          10          20          30
Urine α2U-globulin  E E A S S T R G N L D V A K L N G D W F S I V V A S N K R E K I
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Fig.3. Amino-terminal amino acid sequence of kidney FABP. The sequence of urine α_{2U}-globulin is based on the data reported in [19].

amino acid sequence was determined up to residue 20. The sequence thus determined is: LDVAKLNGDWFSIVVASNKR. A computer search for sequence similarity in the National Biochemical Research Foundation Protein Sequence Database showed that this amino-terminal sequence is identical with the sequence from residues 10 to 29 of urine α_{2U}-globulin [18,19], as shown in fig.3. Furthermore, it was found that the amino acid composition of kidney FABP is very similar to that of the region spanning residues 10–162 (carboxyl-terminus) of urine α_{2U}-globulin (table 1). These results are in agreement with the report of Charles [20] that α_{2U}-globulin in rat kidney lacked the 9 amino-terminal residues. A double-immunodiffusion test using anti-rat kidney FABP antibodies showed further that this protein was present only in male, but not in female, rat kidney (fig.4). This is also consistent with the reported sex-difference in the expression of α_{2U}-globulin [21]. Taken together, it can be concluded that kidney FABP is identical with α_{2U}-globulin from which the 9-residue amino-terminal segment has been removed.

Table 1

Amino acid compositions of P3 protein, kidney FABP and α_{2U}-globulin

Amino acid	mol%		
	P3 protein[a]	Kidney FABP[b]	α_{2U}-Globulin[c]
Asp	13.5	11.7	13.9
Thr	6.0	6.6	6.0
Ser	4.0	4.4	4.0
Glu	11.3	10.8	10.6
Pro	0.9	0.8	0.7
Gly	7.0	7.5	7.3
Ala	3.9	4.4	4.0
Cys	2.0	1.4	2.0
Val	6.1	6.1	6.0
Met	2.5	1.7	2.7
Ile	6.0	6.0	6.6
Leu	9.3	9.1	9.3
Tyr	4.3	4.7	4.6
Phe	6.7	7.3	6.6
Lys	8.7	8.1	8.6
His	2.1	2.2	2.0
Arg	5.1	6.3	5.9
Trp	0.6	0.8	0.7

[a] This study. Data based on an average of three hydrolysates
[b] Data from [10]
[c] Residues 10–162, data from [18,19]

α_{2U}-Globulin is a male-specific rat protein that is synthesized in liver under complex hormonal control, secreted into the blood, and excreted in the urine [22]. Although α_{2U}-globulin is also found in the cytosol of male rat kidney [22], no mRNA for this protein can be detected in the kidney by Northern blot analysis [23]. It is, therefore, likely that urine α_{2U}-globulin is reabsorbed from urine into the renal tubular cells, where it loses its 9-residue amino-terminal segment by proteolytic processing.

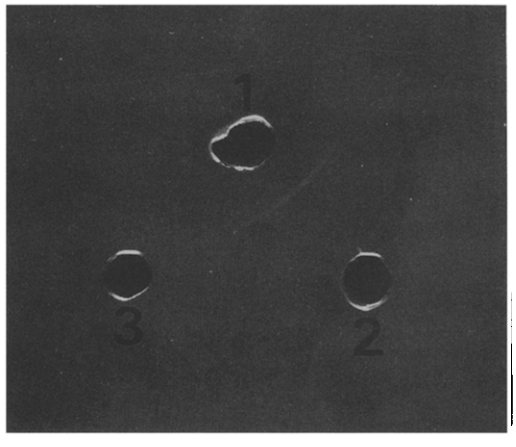

Fig.4. Double-immunodiffusion test. Wells: 1, rabbit antibody to rat kidney FABP; 2, male rat kidney cytosol; 3, female rat kidney cytosol.

α_{2U}-Globulin is a member of the α_{2U}-globulin (ligand-binding carrier) superfamily, which consists of more than a dozen proteins (mostly extracellular) of low molecular masses [24]. Many of the members of this superfamily bind hydrophobic ligands and are thought to be involved in the transport and metabolism of the ligands (e.g. retinol for serum retinol-binding protein and cholesterol for apolipoprotein D). However, no ligands for α_{2U}-globulin have as yet been reported, even though this protein is a representative of this superfamily. The results reported herein indicate that α_{2U}-globulin serves as a carrier of fatty acids in the kidney.

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